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Lipid Composition Analysis of Milk Fats from Different Mammalian Species: Potential for Use as Human Milk Fat Substitutes

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ABSTRACT: The lipid compositions of commercial milks from cow, buffalo, donkey, sheep, and camel were compared with that of human milk fat (HMF) based on total and sn-2 fatty acid, triacylglycerol (TAG), phospholipid, and phospholipid fatty acid compositions and melting and crystallization profiles, and their degrees of similarity were digitized and differentiated by an evaluation model. The results showed that these milk fats had high degrees of similarity to HMF in total fatty acid composition. However, the degrees of similarity in other chemical aspects were low, indicating that these milk fats did not meet the requirements of human milk fat substitutes (HMFSs). However, an economically feasible solution to make these milks useful as raw materials for infant formula production could be to modify these fats, and a possible method is blending of polyunsaturated fatty acids (PUFA) and 1,3-dioleoyl-2-palmitoylglycerol (OPO) enriched fats and minor lipids based on the corresponding chemical compositions of HMF.

KEYWORDS: *human, cow, buffalo, donkey, sheep, camel, mammalian milk fats, chemical composition, human milk fat substitutes, similarity evaluation*

INTRODUCTION

Human milk has a unique composition to provide newborns with all essential nutrients such as protein, fat, carbohydrate, minerals, vitamins, and physiologically active substances.[1,2] Nevertheless, in cases when breastfeeding is not possible, commercial infant formulas are the best alternative to fulfill the nutritional needs of newborns. Commercial infant formulas are usually based on mammalian milks such as cow, buffalo, donkey, sheep, camel, and goat milk. However, these milks are different from human milk in terms of chemical compositions, which may cause nutritional and immunological problems.[3−5]

One of the differences between human milk and other mammalian milks is the milk protein composition. Human milk contains a low content of protein (6−11 g/L) and a low ratio of casein (10−50%) within total protein. Caseins in human milk differ from other mammalian milks in terms of fraction number, amino acid composition, and peptide mappings, which may cause allergic reactions.[3] Furthermore, β-lactoglobulin, a potential allergenic milk component, which is the major whey protein in cow, buffalo, sheep, and donkey milk, is absent in human milk.[6,7] Therefore, many procedures such as heat or enzymatic treatment have been reported to reduce the allergenicity of these milks (clinical manifestations including gastrointestinal, respiratory, cutaneous, and systemic anaphylactic symptoms) before use in infant formula.[8−10]

Besides milk proteins, mammalian milk fat composition also differs from that of human milk fat (HMF). At present, the fats in infant formulas are usually from physical blending of vegetable oils, and their chemical composition and molecular structure are different from those of HMF. Considering large amounts of commercial mammalian milks, it is economical to modify their fats as human milk fat substitutes (HMFSs) for infant formula. However, limited studies have been done so far to systematically compare milk fat composition in human and mammalian milks, not to mention evaluate and modify them. Human milk contains 3−5% of dietary fat, which provides around 50% energy.[11] Saturated fatty acids (palmitic acid) in HMF are mainly located at the sn-2 position; meanwhile, unsaturated fatty acids are esterified at sn-1,3 positions of the glycerol backbone.[12] The triacylglycerol (TAG) species in HMF with this special intermolecular structure are beneficial for the absorption and have great influence on the metabolism of fat in infants.[13−15] HMF, unlike other mammalian milks, contains small amounts of long-chain polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA, n-3), arachidonic acid (AA, n-6), docosapentaenoic acid (DPA, n-3), and eicosapentaenoic acid (EPA, n-3), which influence the development of infants.[16−18] Furthermore, many ruminant milk fats contain short-chain saturated fatty acids (SC-SFA) produced from de novo synthesis within the mammary epithelial cells and trans fatty acids produced from biohydrogenation.[19,20] These fatty acids are almost absent in HMF. The differences in chemical composition between human and other mammalian milk fats may also lead to different physical properties.

This study aimed to compare the lipid compositions of mammalian milks (cow, buffalo, donkey, sheep, and camel milk) with that of human milk on the basis of fatty acid profiles and TAG, phospholipid, and phospholipid fatty acid

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compositions; and their degrees of similarity in lipid compositions were evaluated according to the established model.[21,22] The melting and crystallization profiles of both human and other mammalian milk fats were studied, and the relationship between lipid compositions and physical properties was also elucidated.

MATERIALS AND METHODS

Materials. Forty-five human milk samples were kindly donated by healthy Danish mothers in Aarhus University Hospital (Aarhus, Denmark), who had been well informed before participating in the project. Cow milk (20 samples) was provided by Arla Foods (Aarhus, Denmark). Buffalo milk (20 samples) was purchased from Guiniu Dairy Co., Ltd. (Guangxi, China). Sheep, camel, and donkey milk powder (20 samples, respectively) obtained from spray-drying were purchased from Jiangyulong Biological Technology Co., Ltd. (Xingjiang, China). TAG standards including 1,3-dioleoyl-2-palmitoylglycerol (OPO), 1,2-dioleoyl-3-palmitoylglycerol (OOP), 1,2-dipalmitoyl-3-oleoylglycerol (PPO), 1,3-dipalmitoyl-2-oleoylglycerol (POP), triolein (OOO), 1,2-dioleoyl-3-stearolglycerol (OOS), 1,3-stearol-2-oleoylglycerol (SOS), 1,3-stearol-2-oleoylglycerol (SSO), tripalmitin (PPP), 1-palmitoyl-2-oleoyl-3-stearolglycerol (POS) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden). The phospholipid standards were obtained from Sigma-Aldrich, St. Louis, MO, USA: L-α-phosphatidylethanolamine, dioleoyl (PE; purity 99%), L-α-phosphatidylinositol ammonium salt from soybean (PI; purity 98%), 1,2-diacyl-*sn*-glycero-3-phospho-L-serine from bovine brain (PS; purity 97%), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (PC; purity 99%), and sphingomyelin from bovine brain (SM; purity 97%). Boron trifluoride−methanol solution and silicic acid 60G thin-layer chromatography (TLC) plates were purchased from Sigma-Aldrich. Methanol, hexane, acetonitrile, and isopropanol were all of high-performance liquid chromatography (HPLC) grade purity (Sinopharm Chemical Reagent Co., Ltd., Beijing, China). Hydrochloric acid, glacial acetic acid, and ethyl ether were of analytical grade (Sinopharm Chemical Reagent Co., Ltd.).

Extraction of Total Lipids. Total lipids were extracted from different samples by homogenization with chloroform/methanol (2:1, v/v) as described by Folch et al.[23] The extracted solution was equilibrated by mixing with one-fourth volume of saline solution (NaCl 0.86%, w/w). The solvent phase was filtered and evaporated under vacuum, and the obtained total lipids were stored at −20 °C for further lipid composition analysis.

Fatty Acid Composition. Milk fat was first methylated with methanolic potassium hydroxide as described by Christopherson et al.[24] with slight modification. Fifty milligrams of milk fat was dissolved in 1 mL of hexane and methylated with 50 μL of 2 M methanolic potassium hydroxide. The mixture was vigorously mixed for 30 s, and the supernatant was then analyzed on a gas chromatograph (GC) (Thermo-Fisher Scientific, Waltham, MA, USA) equipped with an autosampler, a flame ionization detector, and an ionic liquid capillary column (Supelco SLB-IL 100, 60 m × 0.25 mm × 0.2 μm, Sigma-Aldrich). Helium was used as the carrier gas with a flow rate of 1 mL/min. The column oven temperature was kept at 170 °C, and the running time for each sample was 60 min. The injection port and detector temperatures were both set at 250 °C. The fatty acid methyl esters were identified by comparing the retention time with the standards, the response factors of the individual fatty acids were calculated relative to the area of palmitic acid, which was assigned a response factor of 1, and the relative contents expressed as mole percent were then calculated.[25]

sn-2 Fatty Acid Composition. sn-2 monoacylglycerols (MAG) were obtained by Grignard degradation with allyl magnesium bromide (AMB) as described by Xu et al.[26] About 30 mg of the sample was dissolved in diethyl ether (10 mL), and 0.3 mL of allyl magnesium bromide was added. After 1 min of vigorous stirring, the reaction was stopped with 8 mL of acid buffer (0.27 M HCl in 0.4 M boric acid). The water phase was removed, and the diethyl ether extract was washed twice with boric acid and dried with anhydrous sodium sulfate. The ether phase was evaporated under nitrogen to 150 μL and separated on the boric acid-impregnated TLC plates with the developing solvent system of chloroform/acetone (90:10, v/v). The band corresponding to sn-2 MAG was scraped off and extracted twice with 2 mL of diethyl ether. Diethyl ether was dried by anhydrous sodium sulfate and evaporated under nitrogen gas. The residue was methylated and analyzed as aforementioned.

TAG Composition. TAG species were analyzed by a reverse-phase high-performance liquid chromatography (RP-HPLC) as previously reported by Zou et al.[22] The evaporative light scattering detector (ELSD) was set at 55 °C at a nitrogen nebulizer gas flow rate of 1.8 mL/min and a gain of 1. The separation was carried out using a Lichrospher C18 column (5 μm, 4.6 × 250 mm; Hanbon Science and Technology Co., Ltd., Jiangsu, China) and eluted with a binary gradient of acetonitrile (A) and isopropanol (B) at a flow rate of 0.8 mL/min with a linear gradient of solvent A from 70 to 60% in the first 30 min, then to 55% in 40 min, and then equilibrated back to 70% for 5 min. The sample concentration was 20 mg/mL, and the injection volume was 10 μL.

TAG identification was carried out on a HPLC−atmospheric pressure chemical ionization mass spectrometr (HPLC-APCI-MS). The MS conditions were as follows: APCI source block and probe temperatures, 100 and 400 °C, respectively; MS multiplier voltage, 700 V; measurement range, m/z 250−1200.

Similarity Evaluation. The fatty acid profiles and TAG, phospholipid, and phospholipid fatty acid compositions of HMF were considered as objectives; that is, the degrees of similarity were 100. The similarity evaluation was carried out by comparison of these chemical indices of different mammalian milk fats with the corresponding indices of HMF. The contents of chemical indices within the corresponding ranges of these of HMF were considered to be identical with HMF. However, the contents of chemical indices outside the ranges have to be evaluated and deducted. On the basis of the "deducting score principle",[21,22] the evaluation model was established as

$$G_m = 100 - \sum_{i=1}^{n} E_{i(m)} \quad (1)$$

$$E_{i(m)} = 100 \times \left(C_{i(m)} \frac{D_{i(m)}}{\sum_{i=1}^{n} D_{i(m)}} \right) \quad (2)$$

$$C_{i(m)} = \frac{|B_{i(m)} - A_{i(m)}|}{A_{i(m)}} \quad (3)$$

where G_m is the degree of similarity of mammalian milk fat to HMF based on total fatty acid composition, relative contents of sn-2 fatty acids, PUFA, TAG, phospholipid composition, fatty acid or PUFA composition of phospholipids; $E_{i(m)}$ is the deducted degree of similarity from the total fatty acid content, relative content of sn-2 fatty acid, PUFA, TAG, phospholipid content, fatty acid or PUFA content of phospholipids that is outside the range of that of HMF; $D_{i(m)}/\Sigma_{i=1}^{n} D_{i(m)}$ is the weight of the total fatty acid, sn-2 fatty acid, PUFA, TAG, phospholipid, fatty acid or PUFA of phospholipids relative to its total amount; $C_{i(m)}$ is the floating coefficient, which is dependent on the total fatty acid content, relative content of sn-2 fatty acid, PUFA, TAG, phospholipid content, fatty acid or PUFA content of phospholipids in mammalian milk fat; $B_{i(m)}$ is the total fatty acid content, relative content of sn-2 fatty acid, PUFA, TAG, phospholipid content, fatty acid or PUFA content of phospholipids in mammalian milk fat; and $A_{i(m)}$ is the upper or lower limit of corresponding total fatty acid content, relative content of sn-2 fatty acid, PUFA, TAG, phospholipid content, fatty acid or PUFA content of phospholipids in HMF. When B is higher than the upper limit of the corresponding fatty acid content, relative content of sn-2 fatty acid, PUFA, TAG, phospholipid content, fatty acid or PUFA content of phospholipids, A is selected as the upper limit, and vice versa. If B is within the range, C is set to zero.

Table 1. Fatty Acid Compositions of Human, Cow, Buffalo, Donkey, Sheep, and Camel Milk Fats[a]

	total fatty acids					
fatty acid (mol %)[b]	human[c]	cow	buffalo	donkey	sheep	camel
C4:0		8.65 ± 0.78b	8.45 ± 0.66b	1.06 ± 0.21a	6.06 ± 0.69b	
C6:0	0.05 ± 0.04a	4.79 ± 0.16c	4.06 ± 0.27c	1.58 ± 0.02b	2.35 ± 0.50b	
C8:0	0.14 ± 0.07a	2.84 ± 0.36bc	2.09 ± 1.09b	1.51 ± 0.06b	3.95 ± 0.35c	
C10:0	1.71 ± 1.35a	4.69 ± 0.44b	2.65 ± 0.46ab	2.92 ± 0.49ab	9.78 ± 1.19c	
C12:0	6.74 ± 2.54b	3.90 ± 0.29ab	2.74 ± 0.46a	2.89 ± 1.09a	4.10 ± 0.57ab	1.05 ± 0.07a
C14:0	8.54 ± 2.83ab	11.76 ± 1.93ab	10.84 ± 1.04ab	7.52 ± 0.61a	9.37 ± 1.19ab	11.84 ± 0.29c
C14:1 ω-5	0.32 ± 0.15a	0.62 ± 0.12ab		0.75 ± 0.20b	0.81 ± 0.16b	0.72 ± 0.11ab
C16:0	23.83 ± 3.43a	30.43 ± 0.80bcd	34.64 ± 1.92d	31.24 ± 1.67cd	25.35 ± 1.45ab	27.07 ± 0.73abc
C16:1 ω-7	2.00 ± 0.50a	1.88 ± 0.17a	3.59 ± 0.58b	1.95 ± 0.87a	1.09 ± 0.13a	9.74 ± 0.51c
C18:0	6.09 ± 1.09a	7.50 ± 0.71ab	8.10 ± 0.35ab	8.31 ± 1.71ab	9.64 ± 0.69ab	11.85 ± 0.93b
C18:1t		1.55 ± 0.35ab	1.65 ± 0.21b		1.25 ± 0.21ab	0.95 ± 0.07a
C18:1 ω-9	33.43 ± 5.18ab	17.94 ± 0.71a	18.00 ± 0.85a	31.77 ± 0.50b	21.54 ± 0.76a	29.25 ± 1.77ab
C18:2t		0.37 ± 0.10b	0.10 ± 0.02a		0.25 ± 0.07ab	0.18 ± 0.04a
C18:2 ω-6	10.57 ± 4.96b	1.12 ± 0.17a	1.52 ± 0.19a	5.53 ± 0.81ab	2.62 ± 0.27a	3.31 ± 0.27a
C20:0	0.25 ± 0.13a	0.36 ± 0.08a	0.25 ± 0.08a	0.16 ± 0.05a	0.20 ± 0.05a	0.62 ± 0.11b
C18:3 ω-6	0.05 ± 0.04a		0.09 ± 0.04ab	0.15 ± 0.02ab	0.13 ± 0.03ab	0.17 ± 0.05c
C20:1 ω-9	0.24 ± 0.15a		0.34 ± 0.10a	0.42 ± 0.15a	0.14 ± 0.04a	0.14 ± 0.02a
C18:3 ω-3	0.67 ± 0.17bc	0.13 ± 0.03a	0.42 ± 0.13ab	0.51 ± 0.48ab	1.11 ± 0.29 cd	1.37 ± 0.05d
C20:2 ω-6	0.42 ± 0.24a	0.12 ± 0.04a				
C20:3 ω-6	0.42 ± 0.19a	0.42 ± 0.08a	0.17 ± 0.07a	0.23 ± 0.04a		0.36 ± 0.06a
C20:4 ω-6	0.45 ± 0.13a	0.05 ± 0.01b				
C20:5 ω-3	0.17 ± 0.04					
C22:0	0.13 ± 0.05a	0.07 ± 0.03a	0.12 ± 0.01a			
C22:1 ω-9	0.16 ± 0.10a					
C22:2 ω-6	0.08 ± 0.03a	0.03 ± 0.02a				
C24:0	0.09 ± 0.03ab	0.05 ± 0.02a	0.04 ± 0.02b	0.17 ± 0.04c	0.17 ± 0.03c	
C24:1 ω-9	0.21 ± 0.13a					
C22:4 ω-6	0.17 ± 0.11a					
C22:5 ω-6	0.15 ± 0.06a					
C22:5 ω-3	0.28 ± 0.09a					
C22:6 ω-3	0.51 ± 0.23a					
SFA	49.29 ± 5.74a	75.49 ± 3.84b	73.99 ± 4.50b	57.96 ± 2.20a	70.97 ± 5.22b	53.66 ± 4.85a
SC-SFA		8.65 ± 0.78b	8.45 ± 0.66b	1.06 ± 0.21a	6.06 ± 0.69b	
MC-SFA	8.86 ± 4.52b	16.22 ± 1.25 cd	11.55 ± 1.73bc	8.91 ± 1.62b	20.17 ± 2.61d	1.05 ± 0.07a
LC-SFA	40.43 ± 5.02a	50.62 ± 2.03bc	53.99 ± 2.19c	47.99 ± 0.63bc	44.74 ± 3.23ab	52.61 ± 0.92c
MUFA	36.90 ± 5.95b	21.63 ± 1.70a	23.57 ± 0.37a	35.01 ± 1.49b	24.84 ± 0.80a	41.00 ± 1.20b
PUFA	12.65 ± 1.32c	1.89 ± 0.18a	2.30 ± 0.40a	6.05 ± 1.29b	4.19 ± 0.73ab	5.21 ± 0.34b
PUFA ω-3	1.70 ± 0.43c	0.52 ± 0.13a	0.42 ± 0.06ab	0.35 ± 0.07a	1.20 ± 0.32bc	0.36 ± 0.06a
LC-PUFA ω-3	1.02 ± 0.39a					
PUFA ω-6	10.95 ± 1.34d	1.87 ± 0.17a	1.78 ± 0.50a	6.05 ± 1.29c	2.84 ± 0.37ab	5.04 ± 0.37bc
LC-PUFA ω-6	2.07 ± 0.90b	0.62 ± 0.03a	0.17 ± 0.07a		0.19 ± 0.07a	0.36 ± 0.06a
ω-6/ω-3	6.77 ± 2.68b	3.70 ± 0.62ab	4.24 ± 1.19ab	16.50 ± 0.85c	2.41 ± 0.32a	14.19 ± 1.33c

[a]Means ± SD with the same letter are not significantly different at the 0.05 probability level. [b]SFA, saturated fatty acids; SC-SFA, short-chain SFA; MC-SFA, medium-chain SFA; LC-SFA, long-chain SFA; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain PUFA; ω-6/ω-3, PUFA ω-6/PUFA ω-3. [c]Data were cited and reorganized from the results reported by Zou et al.[25]

Polar Lipid Composition. Analysis of polar lipids was carried out on an HPLC equipped with an ELSD as previously described by Ménard.[27] Nitrogen was used as the nebulizing gas at a flow rate of 1 L/min, and the evaporating temperature was set at 85 °C. A silica column (5 μm, 4.6 × 250 mm; Hanbon Science and Technology Co., Ltd., Jiangsu, China) conjugated with a precolumn with the same packing and internal diameter was used. The elution program was isocratic conditions with 87.5:12:0.5 (v/v/v) choloroform/methanol/triethylamine buffer (pH 3, 1 M formic acid) from 0 to 10 min and then a linear gradient with 87.5:12:0.5 (v/v/v) at t = 11 min to 28:60:12 (v/v/v) at t = 45 min. The mobile phase was brought back to the initial conditions at t = 47 min, and the column was allowed to equilibrate until the next injection at t = 55 min. The flow rate was maintained at 0.5 mL/min, the injection volume was 10 μL, and the samples and the column were equilibrated at 40 °C. The identification of polar lipids was carried out by comparison with the retention time of standards, and the quantitation of polar lipids was performed as in our previously reported study.[25]

Fatty Acid Composition of Polar Lipids. The polar lipids were separated from the total lipids by silicic acid 60G TLC plates with the developing solvent system of hexane/diethyl ether/acetic acid (80:20:1, v/v/v). The polar lipids were scraped off and extracted with 3 mL of a chloroform/methanol/water (5:5:1, v/v/v) mixture.[28] After centrifugation at 2773g for 10 min, the organic phase was collected. The remaining water phase was extracted twice with the same method, and the organic solvent was pooled and evaporated. Three hundred microliters of BF_3 methanol solution was added for methylation, and the screw-capped tubes were kept at 100 °C for 90 min. Six hundred microliters of heptane and 500 μmol of saturated NaCl solution were added. The mixture was centrifuged at 2773g for

Table 2. sn-2 Fatty Acid Compositions of Human, Cow, Buffalo, Donkey, Sheep, and Camel Milk Fats[a]

	sn-2 fatty acids					
fatty acid (mol %)[b]	human[c]	cow	buffalo	donkey	sheep	camel
C4:0		4.16 ± 0.62a	3.28 ± 0.44a		4.47 ± 0.49a	
C6:0	0.07 ± 0.04a	3.10 ± 0.22c	2.26 ± 0.15b		2.43 ± 0.53bc	
C8:0	0.20 ± 0.11a	2.35 ± 0.23d	1.23 ± 0.22bc	1.01 ± 0.19ab	2.17 ± 0.68 cd	
C10:0	0.79 ± 0.68a	4.90 ± 0.53b	1.86 ± 0.24a	4.87 ± 0.35b	11.11 ± 1.82c	
C12:0	6.26 ± 3.44b	6.52 ± 0.50b	3.13 ± 0.34ab	4.29 ± 0.48ab	5.81 ± 0.06b	0.91 ± 0.18a
C14:0	13.08 ± 5.04ab	20.76 ± 1.72c	16.62 ± 0.65bc	14.44 ± 0.34ab	8.27 ± 1.51a	14.38 ± 0.18ab
C14:1 ω-5	0.44 ± 0.32a	1.55 ± 0.29b	2.58 ± 0.58c	1.30 ± 0.21ab		
C16:0	52.66 ± 3.91d	32.03 ± 2.95ab	39.23 ± 0.71c	27.12 ± 2.18a	27.60 ± 1.60a	35.53 ± 0.48bc
C16:1 ω-7	1.91 ± 0.85a	2.35 ± 0.21a	4.13 ± 0.41b	1.79 ± 0.08a	3.83 ± 0.44b	10.72 ± 0.27c
C18:0	1.72 ± 0.58a	4.10 ± 0.39bc	4.97 ± 0.56c	4.95 ± 0.16c	5.26 ± 0.75c	3.02 ± 0.28ab
C18:1t		1.10 ± 0.13a				
C18:1 ω-9	9.99 ± 3.88a	13.21 ± 0.55ab	15.70 ± 0.78bc	30.71 ± 0.18d	19.85 ± 2.25c	27.63 ± 0.66d
C18:2t		0.31 ± 0.05a	0.08 ± 0.02b			
C18:2 ω-6	6.85 ± 4.20b	2.07 ± 0.02a	1.98 ± 0.21a	7.57 ± 0.12b	4.96 ± 0.53ab	4.24 ± 0.04ab
C20:0	0.40 ± 0.20b		0.10 ± 0.02a	0.11 ± 0.03ab	0.33 ± 0.10ab	
C18:3 ω-6	0.04 ± 0.02a					
C20:1 ω-9	0.13 ± 0.03ab	0.50 ± 0.09c	0.28 ± 0.04b	0.04 ± 0.01a	0.49 ± 0.11c	
C18:3 ω-3	0.50 ± 0.35ab	0.14 ± 0.04a	0.54 ± 0.07ab	0.63 ± 0.08b	0.74 ± 0.12b	0.61 ± 0.05b
C20:2 ω-6	0.19 ± 0.05a					
C20:3 ω-6	0.22 ± 0.15a	0.21 ± 0.10a	0.09 ± 0.04a	0.10 ± 0.03a		
C20:4 ω-6	0.42 ± 0.39a					
C20:5 ω-3	0.29 ± 0.20a					
C22:0	0.15 ± 0.12a	0.06 ± 0.02a				
C22:1 ω-9	0.10 ± 0.05a	0.06 ± 0.02a				
C22:2 ω-6	0.20 ± 0.14a	0.04 ± 0.04a				
C24:0	0.09 ± 0.03a	0.07 ± 0.01a				
C24:1 ω-9	0.42 ± 0.22a					
C22:4 ω-6	0.21 ± 0.16a					
C22:5 ω-6	0.27 ± 0.12a					
C22:5 ω-3	0.47 ± 0.16a					
C22:6 ω-3	0.65 ± 0.24a					
SFA	75.65 ± 7.65cd	78.33 ± 3.15d	73.73 ± 0.17cd	57.45 ± 0.45ab	67.45 ± 3.87bc	54.95 ± 0.29a
SC-SFA		4.16 ± 0.62a	3.28 ± 0.44a		4.47 ± 0.49a	
MC-SFA	7.48 ± 4.94ab	16.88 ± 1.78 cd	8.48 ± 0.65b	10.16 ± 1.01bc	21.52 ± 3.09d	0.91 ± 0.18a
LC-SFA	68.18 ± 4.52d	57.30 ± 1.51bc	61.97 ± 0.38 cd	47.28 ± 1.46ab	41.46 ± 7.05a	54.04 ± 0.11bc
MUFA	13.70 ± 4.03a	18.90 ± 1.26ab	23.05 ± 0.40bc	34.15 ± 0.17d	25.92 ± 3.05c	38.54 ± 0.36d
PUFA	10.64 ± 3.70c	3.02 ± 0.32a	2.80 ± 0.37a	8.29 ± 0.26bc	5.89 ± 1.30ab	6.27 ± 0.01abc
PUFA ω-3	2.11 ± 0.94c	0.32 ± 0.16a	0.75 ± 0.14a	0.73 ± 0.14a	0.93 ± 0.28ab	2.03 ± 0.03bc
LC-PUFA ω-3	1.60 ± 0.78b					
PUFA ω-6	8.53 ± 3.22c	2.57 ± 0.17a	2.06 ± 0.25a	7.66 ± 0.18bc	4.96 ± 1.03abc	4.24 ± 0.04ab
LC-PUFA ω-6	1.62 ± 1.12b	0.24 ± 0.13a	0.09 ± 0.04a	0.10 ± 0.03a		
ω-6/ω-3	3.73 ± 1.88ab	8.98 ± 3.99bc	2.76 ± 0.17a	10.72 ± 1.87c	5.40 ± 0.50abc	2.09 ± 0.05a

[a]Means ± SD with the same letter are not significantly different at the 0.05 probability level. [b]SFA, saturated fatty acids; SC-SFA, short-chain SFA; MC-SFA, medium-chain SFA; LC-SFA, long-chain SFA; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain PUFA. ω-6/ω-3, PUFA ω-6/PUFA ω-3. [c]Data were cited and reorganized from the results reported by Zou et al.[25]

10 min, and the solvent phase was collected and dried by anhydrous sodium sulfate. After centrifugation, the upper layer was injected into GC for fatty acid analysis as aforementioned.

Melting and Crystallization Profiles. The melting and crystallization properties of different mammalian milk fats were determined by a differential scanning calorimeter (DSC Q2000, TA Instruments, Leatherhead, UK), as described by Teichert et al.[29] Calibration was performed using indium and octadecane. Sample weighing from 5 to 10 mg was sealed in an aluminum pan with an empty pan as a reference. The sample was heated from 25 to 80 °C at 50 °C/min and kept at 80 °C for 10 min, then cooled to −55 °C at 10 °C/min, held at −55 °C for 10 min, and finally heated to 80 °C at 5 °C/min. The thermographs were analyzed using Thermal Solutions software (TA Instruments).

Statistical Analysis. All indices of each sample were analyzed three times, and the data were subjected to analysis of variance (ANOVA) using Statistical Analysis System software (SAS, Cary, NC, USA). The significance level being tested was $\alpha = 0.05$, and differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Fatty Acid Composition and Positional Distribution. Fatty acid compositions of human and other mammalian milk fats are presented in Table 1. The saturated fatty acid (SFA) contents in cow, buffalo, and sheep milk fats were significantly higher ($P < 0.001$) than that in HMF. Cows, buffalos, and sheep are ruminants with high contents of SC-SFA (butyric

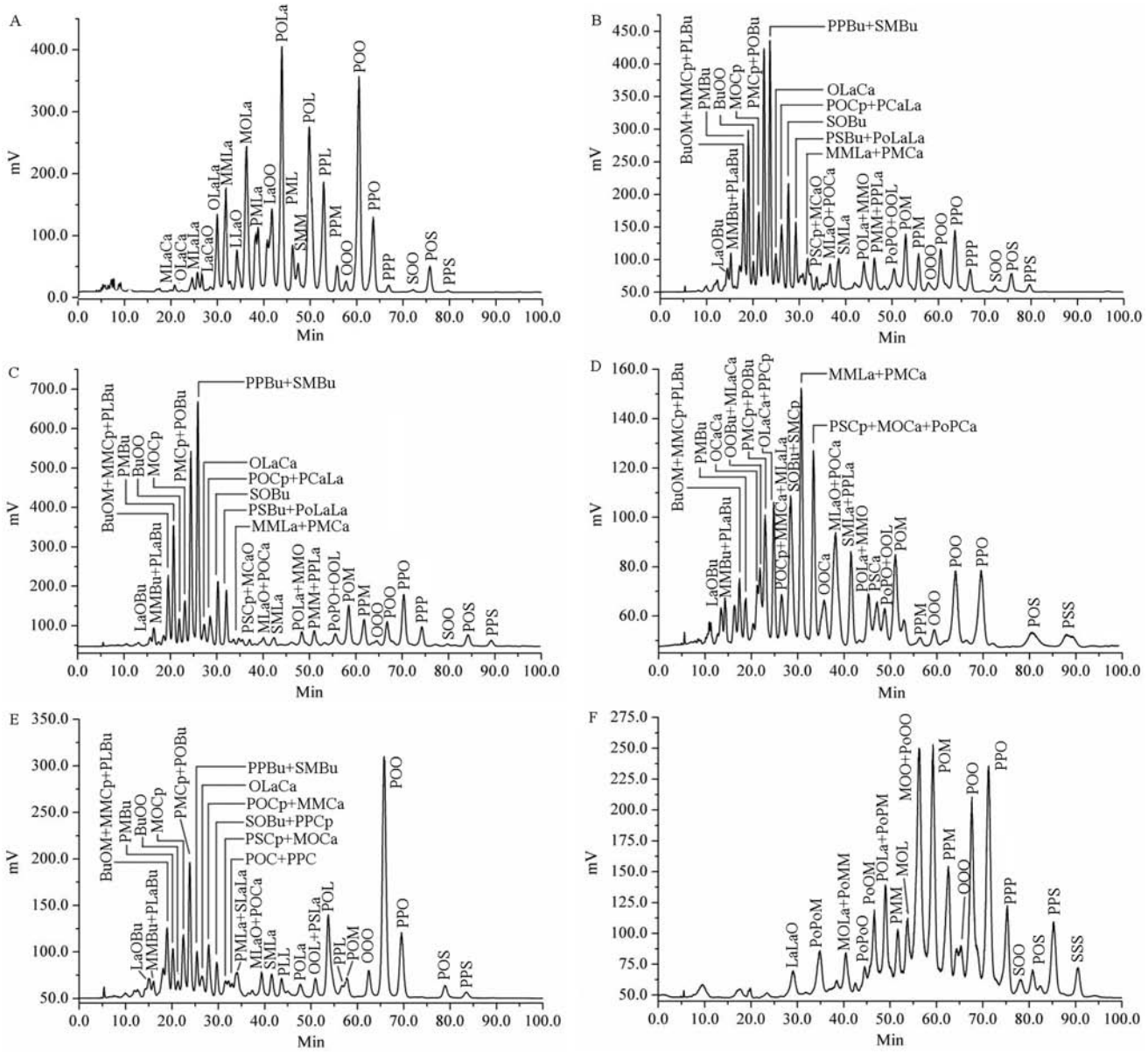

Figure 1. RP-HPLC chromatograms of TAG species in human (A), cow (B), buffalo (C), sheep (D), donkey (E), and camel (F) milk fats. The chromatogram of human milk fat was cited from our previously reported results.[22].

acid) and medium-chain saturated fatty acids (MC-SFA; caproic, caprylic, capric, lauric acid) in their milk. However, camels are also ruminants, yet there are no SC-SFA and few MC-SFA in its milk fat, which was in accordance with a previously reported study.[30] Donkeys have a large cecum, which is responsible for a majority of the fermentation. Therefore, even though donkeys are not ruminants, some SC-SFA was also detected in their milk fat. In terms of long-chain saturated fatty acid (LC-SFA), the contents in cow, buffalo, donkey, and camel milk fats were significantly higher ($P < 0.001$) than that in HMF, which was because cow, buffalo, and donkey milk fats had higher ($P < 0.001$) contents of palmitic acid (C16:0) and camel milk fat had higher ($P < 0.001$) contents of myristic acid (C14:0) and strearic acid (C18:0). The contents of monounsaturated fatty acids (MUFA) in cow, buffalo, and sheep milk fats were significantly lower ($P < 0.001$) than that in HMF, whereas donkey and camel milk fats had almost similar ($P > 0.05$) MUFA contents, which was because donkey milk fat had a high content of oleic acid (C18:1) and camel milk fat had high contents of palmitoleic acid (C16:1) and C18:1. With regard to polyunsaturated fatty acids (PUFA), the contents in cow, buffalo, donkey, sheep, and camel milk fats were significantly lower ($P < 0.001$) than that in HMF, which was mainly due to the significantly higher ($P < 0.001$) content of linoleic acid (C18:2 ω-6) in HMF. Compared with HMF, cow, buffalo, sheep, and camel milk fats were low in long-chain polyunsaturated fatty acid (LC-PUFA) ω-6 contents. Meanwhile, no LC-PUFA ω-3 was detected in these milk fats. The balance between ω-6 and ω-3 fatty acids is of great importance to the development of infants. As shown in Table 1, the ratios in donkey, sheep, and camel milk fats were 16.50 ± 0.85, 2.41 ± 0.32, and 14.19 ± 1.33, respectively, which were significantly different from that of HMF (6.77 ± 2.68). From the view of fatty acid composition, cow, buffalo, donkey, sheep, and camel milk fats were, to some degree, different from HMF. However, by addition of other vegetable oils, the fatty acid composition of

Table 3. TAG Composition of Human, Cow, Buffalo, Sheep, Donkey, and Camel Milk Fats[a]

TAG[b]	human[c]	cow	buffalo	sheep	donkey	camel
LaOBu		1.55 ± 0.08b	0.78 ± 0.09a	1.46 ± 0.12b	1.66 ± 0.14b	
MMBu + PLaBu		1.58 ± 0.10b	1.39 ± 0.12ab	1.58 ± 0.11b	1.18 ± 0.19a	
BuOM + MMCp + PBuL		5.39 ± 0.11c	5.10 ± 0.30bc	2.13 ± 0.17a	4.79 ± 0.13b	
PMBu		6.19 ± 0.12c	7.71 ± 0.25d	1.76 ± 0.07a	2.95 ± 0.08b	
OCaCa				1.74 ± 0.12a		
BuOO		1.52 ± 0.11ab	1.92 ± 0.08b	2.52 ± 0.16c	1.33 ± 0.31a	
MOCp		4.19 ± 0.15b	3.37 ± 0.15a	—	4.43 ± 0.20b	
PMCp + POBu		11.61 ± 0.69c	13.14 ± 0.51d	5.09 ± 0.11a	7.78 ± 0.18b	
PPBu + SMBu		10.63 ± 0.81b	16.02 ± 1.27c		2.95 ± 0.20a	
MCaLa	0.19 ± 0.14a					
OLaCa	0.58 ± 0.22a	1.98 ± 0.14c	1.22 ± 0.16b	4.68 ± 0.15d	2.00 ± 0.16c	
MLaLa	0.54 ± 0.45a					
POCp + PCaLa		4.29 ± 0.31b	2.97 ± 0.19a	2.58 ± 0.13a	3.77 ± 0.14b	
LaLaO	2.58 ± 1.03a					1.75 ± 0.19a
SOBu		4.42 ± 0.36b	4.44 ± 0.31b	7.70 ± 0.32c	2.38 ± 0.23a	
PSBu + PoLaLa		3.29 ± 0.17a	4.55 ± 0.28b			
MMLa + PMCa	1.85 ± 1.49a	1.85 ± 0.12a	0.81 ± 0.10a	9.53 ± 0.57b		
PSCp + MCaO		0.87 ± 0.07b	0.49 ± 0.11a	7.54 ± 0.21d	1.34 ± 0.07c	
POC + PPC					1.15 ± 0.18a	
LLaO	1.99 ± 0.61a					
PMLa + SLaLa	2.42 ± 1.41a				2.78 ± 0.15a	
PoPoM						2.89 ± 0.17a
OOCa				3.53 ± 0.22a		
MLaO + POCa	6.65 ± 2.40b	2.25 ± 0.23a	1.46 ± 0.21a	7.66 ± 0.44b	2.45 ± 0.15a	2.25 ± 0.11a
SMLa		2.46 ± 0.12b	1.18 ± 0.18a	4.17 ± 0.19c	2.08 ± 0.18b	
LaOO	6.54 ± 2.21a					
PML	1.77 ± 1.17a					
PLL					1.78 ± 0.13a	
PoPoO						1.43 ± 0.18a
PoOM						4.68 ± 0.12a
POLa + MMO	10.39 ± 3.02c	2.61 ± 0.16ab	1.78 ± 0.17a	2.98 ± 0.24ab	1.49 ± 0.21a	5.68 ± 0.14b
PSCa				2.62 ± 0.16a		
PMM + PPLa		2.51 ± 0.30b	1.58 ± 0.32a			3.55 ± 0.17c
PoPO + OOL		2.48 ± 0.11c	1.96 ± 0.12ab	2.28 ± 0.14bc	1.77 ± 0.12a	
SMM	2.56 ± 1.35a					
POL	16.93 ± 3.27a				8.54 ± 0.11b	
MOL						3.71 ± 0.23a
MOO + PoOO						13.67 ± 1.29a
PPL	7.15 ± 1.06a				1.15 ± 0.22b	
POM		5.04 ± 0.38b	5.25 ± 0.36b	5.48 ± 0.18b	2.17 ± 0.14a	12.78 ± 1.04c
PPM	1.35 ± 0.56a	2.67 ± 0.19b	3.40 ± 0.30b	0.88 ± 0.15a		6.76 ± 0.31c
OOO	2.04 ± 0.98ab	1.16 ± 0.13a	1.22 ± 0.15a	1.15 ± 0.11a	2.70 ± 0.05b	2.52 ± 0.36b
POO	21.52 ± 5.39c	4.16 ± 0.19a	3.60 ± 0.22a	6.17 ± 0.1ab4	21.35 ± 1.45c	10.67 ± 0.23b
PPO	6.41 ± 1.38a	5.43 ± 0.39a	6.15 ± 0.19a	5.45 ± 0.25a	6.13 ± 0.34a	11.77 ± 0.46b
PPP	0.45 ± 0.22a	1.82 ± 0.12b	2.67 ± 0.23c			4.21 ± 0.13d
SOO	0.59 ± 0.24a	0.76 ± 0.15a	0.33 ± 0.11a			1.31 ± 0.15b
POS	2.28 ± 0.58b	1.57 ± 0.10ab	1.58 ± 0.10ab	2.35 ± 0.29b	1.30 ± 0.26a	1.44 ± 0.17a
PPS	0.14 ± 0.06a	0.66 ± 0.05b	0.92 ± 0.07b	1.70 ± 0.16c	0.83 ± 0.06b	4.23 ± 0.20d
SSS						1.76 ± 0.16a

[a]Means ± SD with the same letter are not significantly different at the 0.05 probability level. [b]Bu, butyric acid; Cp, caproic acid; C, caprylic acid; Ca, capric acid; La, lauric acid; M, myristic acid; P, palmitic acid; Po, pamitoleic acid; S, strearic acid; O, oleic acid; L, linoleic acid. [c]Data were cited and reorganized from the results reported by Zou et al.[22]

these milk fats can be adjusted to mimic HMF, and this method is also currently most commonly used by infant formula producers.

HMF has a special fatty acid distribution with most of the SFA located at the sn-2 position and UFA at the sn-1,3 positions, which is of great importance for the digestion, absorption, and subsequent metabolism in infants. The compositions of sn-2 fatty acids of cow, buffalo, donkey, sheep, and camel milk fats were analyzed and compared with HMF (Table 2). Compared with the content of SFA at the sn-2 position in HMF, cow, buffalo, and sheep milk fats had similar contents ($P > 0.05$), whereas the contents in donkey and camel milk fats were significantly lower ($P < 0.001$). However, in cow and sheep milk fats, MC-SFA accounted for a high proportion

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Table 4. Concentration of Polar Lipids of Human, Cow, Buffalo, Sheep, Donkey, and Camel Milk Fats and Relative Proportion of Each Class of Polar Lipids[a]

polar lipid[b]	human[c]	cow	buffalo	sheep	donkey	camel
Content of Polar Lipids (Milligrams of Polar Lipids per Total Lipids)						
PE	0.65 ± 0.20a	1.45 ± 0.19bc	1.03 ± 0.12ab	1.54 ± 0.16c	1.23 ± 0.17bc	1.65 ± 0.18c
PI	0.39 ± 0.03c	0.47 ± 0.02d	0.13 ± 0.01a	0.15 ± 0.02a	0.17 ± 0.01a	0.28 ± 0.02b
PS	0.74 ± 0.15c	0.35 ± 0.03b	0.12 ± 0.01a	0.14 ± 0.01a	0.16 ± 0.01a	0.22 ± 0.03ab
PC	1.28 ± 0.22a	1.20 ± 0.04a	0.96 ± 0.10a	1.27 ± 0.15a	1.01 ± 0.13a	1.19 ± 0.12a
SM	2.05 ± 0.28c	1.31 ± 0.11ab	1.01 ± 0.08a	1.19 ± 0.10ab	1.44 ± 0.11b	1.31 ± 0.16ab
total	5.11 ± 0.72c	4.78 ± 0.22bc	3.22 ± 0.19a	4.30 ± 0.23bc	4.01 ± 0.26ab	4.65 ± 0.31bc
Relative Proportion of Polar Lipids (Percent of Polar Lipids)						
PE	12.48 ± 2.93a	30.23 ± 2.69b	31.10 ± 1.41b	35.85 ± 1.77b	30.60 ± 2.12b	35.50 ± 1.41b
PI	7.69 ± 0.75c	9.89 ± 0.87d	3.95 ± 0.35a	3.45 ± 0.64a	4.20 ± 0.57ab	6.05 ± 0.92bc
PS	14.36 ± 2.02c	7.32 ± 0.99b	3.60 ± 0.42a	3.35 ± 0.49a	4.00 ± 0.42a	4.75 ± 0.21b
PC	25.08 ± 3.71a	25.20 ± 1.88a	29.75 ± 1.34a	29.60 ± 1.56a	25.25 ± 1.48a	25.55 ± 1.91a
SM	40.18 ± 1.14c	27.36 ± 1.07a	31.60 ± 1.98ab	27.75 ± 0.92a	35.95 ± 2.62bc	28.15 ± 1.63a

[a]Mean ± SD with the same letter are not significantly different at the 0.05 probability level. [b]PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin. [c]Data were cited and reorganized from the results reported by Zou et al.[25]

in SFA and thus the contents of long-chain saturated fatty acids (LC-SFA) were significantly lower ($P < 0.001$). Buffalo milk fat had a similar content of LC-SFA to HMF, but the content of C18:0 was significantly higher ($P < 0.01$). The fatty acids composed of SFA at the sn-2 position in HMF were mainly C16:0, whereas the contents of C16:0 in cow, buffalo, donkey, sheep, and camel milk fats were significantly lower ($P < 0.001$). Furthermore, in terms of relative content of C16:0 at the sn-2 position, calculated as 100% × sn-2 C16:0/(3 × total C16:0), the values of cow, buffalo, donkey, sheep, and camel milk fats were much less than that in HMF. Due to the lower contents of SFA at the sn-2 position in donkey and camel milk fats, they had higher contents of UFA than that HMF. In terms of milk fats with similar contents of SFA at the sn-2 position to HMF, cow, buffalo, and sheep milk fats had significantly higher ($P < 0.001$) contents of MUFA, and at the same time, their PUFA contents were significantly lower ($P < 0.001$). Other characteristics with regard to sn-2 fatty acids of cow, buffalo, donkey, sheep, and camel milk fats such as LC-PUFA, ω-6/ω-3, can be seen in Table 2. Therefore, in terms of fatty acid distribution, these mammalian milk fats had low degrees of similarity to HMF.

TAG Composition. HMF is ingested in the form of TAG, and newborn infants, especially preterm infants, have a reduced digestive ability due to their low levels of pancreatic lipase and bile slats.[31,32] Thus, on the basis of the principle that HMF was the golden rule for HMFS production, the best index for HMFS evaluation should be TAG composition.[22] In this study, the TAG compositions of cow, buffalo, donkey, sheep, and camel milk fats were analyzed and compared with that of HMF, and the chromatographs with identified peaks are shown in Figure 1; TAG compositions are presented in Table 3. With regard to TAG species, cow, buffalo, sheep, and camel milk fats contained more TAG species than those HMF due to higher amounts of SC- and MC-SFA, and these TAGs, such as LaOBu, MMBu, PLaBu, BuOM, PMBu, OCaCa, BuOO, MOCp, PMCp, SMBu, MCaLa, OLaCa, and MLaLa, had low equivalent carbon numbers (ECN), which were therefore eluted out in the front of the chromatograms (Figure 1). These results were in accordance with the previous study.[33] Camel milk fat was free of SC-SFA and also had lower percentages of MC-SFA, and thus the TAGs in this milk fat mostly composed of long-chain fatty acids had higher ECN, but fewer TAG species. Similar results were also reported by Haddad et al.[34] HMF contained higher amounts of TAG with palmitic acid, such as PPO, POO, PPL, POL, and POLa. Compared with HMF, cow, buffalo, sheep, and donkey milk fats had similar ($P > 0.05$) contents of PPO, whereas camel milk fat had a significantly higher ($P < 0.001$) content. In terms of POO, cow, buffalo, sheep, and camel milk fat had significantly lower ($P < 0.001$) contents than HMF, whereas donkey milk fat had a similar ($P > 0.05$) content. With regard to POL and PPL, the contents in cow, buffalo, sheep, and camel milk fats were too low to be detected, and the contents in donkey milk fat were significantly lower ($P < 0.001$). The contents of POLa in milk fats from cow, buffalo, sheep, donkey, and camel were significantly lower ($P < 0.001$) than that in HMF. Due to the different locations of fatty acids on three positions of the glycerol backbone, TAGs with the same fatty acid composition had different isomers. However, it could be speculated that most of the palmitic acid in cow, buffalo, sheep, donkey, and camel milk fats was located at sn-1,3 positions due to low relative contents of palmitic acid at the sn-2 position.

Phospholipids and Their Fatty Acid Composition. Phospholipids compose the backbone of the milk fat globule membrane and play great roles in the development of infants.[35] The choline-containing polar lipids such as SM and PC are of particular importance because choline is required for rapid organ growth and membrane biosynthesis in neonates.[36] The contents and relative proportions of polar lipids in cow, buffalo, donkey, sheep, and camel milk fats were analyzed and compared with these of HMF (Table 4). The contents of total polar lipids in buffalo and donkey milk fats were significantly lower ($P < 0.001$) than that in HMF, whereas no significant difference ($P > 0.05$) was found in cow, sheep, and camel milk fats. By analysis of the polar lipid contents of bovine milk fat globules with different sizes, Lopez et al. concluded that the contents of polar lipids were negatively correlated with the diameters of milk fat globules.[37] Therefore, on the basis of the contents of polar lipids, it could be deduced that the milk fat globules are expected to have a descending order in size from buffalo, donkey, sheep, camel, and cow to human. In terms of the composition of polar lipids, the contents of SM in cow, buffalo, donkey, sheep, and camel milk

Table 5. Fatty Acid Compositions of Phospholipids in Human, Cow, Buffalo, Donkey, Sheep, and Camel Milk Fats[a]

fatty acid (mol %)[b]	human[c]	cow	buffalo	donkey	sheep	camel
C4:0		1.95 ± 0.16a	2.19 ± 0.45a		1.81 ± 0.35a	
C6:0	0.06 ± 0.02a	1.18 ± 0.22b	0.71 ± 0.33b		1.01 ± 0.12b	
C8:0	0.35 ± 0.19a	1.27 ± 0.11b	0.57 ± 0.27a	0.74 ± 0.42ab	0.89 ± 0.14ab	
C10:0	0.47 ± 0.35a	2.38 ± 0.04b	0.72 ± 0.12a	0.73 ± 0.20a	0.96 ± 0.05a	
C12:0	1.38 ± 0.68a	3.43 ± 0.27b	2.76 ± 0.20b	0.74 ± 0.23a	0.78 ± 0.07a	
C14:0	8.30 ± 2.92b	5.94 ± 0.20ab	7.30 ± 0.84b	3.43 ± 0.17a	3.17 ± 0.12a	3.49 ± 0.52a
C14:1 ω-5	0.54 ± 0.24a	0.33 ± 0.05a	0.56 ± 0.04a	0.36 ± 0.03a	0.44 ± 0.04a	0.38 ± 0.05a
C16:0	39.86 ± 7.03c	17.17 ± 1.95a	35.19 ± 1.40c	26.67 ± 0.67b	21.45 ± 2.14ab	19.76 ± 0.55ab
C16:1 ω-7	0.58 ± 0.50a	1.82 ± 0.02b	1.72 ± 0.28b			3.70 ± 0.35c
C18:0	13.08 ± 2.95b	15.40 ± 0.29b	9.34 ± 0.54a	13.68 ± 0.56b	14.13 ± 1.10b	16.01 ± 1.04b
C18:1t		0.60 ± 0.06a	1.26 ± 0.30b		2.81 ± 0.26c	2.84 ± 0.38c
C18:1 ω-9	13.14 ± 2.63a	30.01 ± 1.18c	23.35 ± 1.98b	29.08 ± 1.43c	36.08 ± 2.46d	25.87 ± 1.32bc
C18:2t		0.38 ± 0.12a			0.19 ± 0.04a	
C18:2 ω-6	12.87 ± 4.24b	6.77 ± 0.17a	8.14 ± 0.28a	17.06 ± 0.40b	8.54 ± 0.70a	13.25 ± 0.74b
C20:0	0.38 ± 0.15ab	0.31 ± 0.08a	0.65 ± 0.08b	0.43 ± 0.09ab	0.49 ± 0.10ab	0.53 ± 0.11ab
C18:3 ω-6	0.04 ± 0.02a	0.18 ± 0.10a	0.58 ± 0.03b		1.30 ± 0.14c	
C20:1 ω-9	0.57 ± 0.24a	0.75 ± 0.06a		0.51 ± 0.09a		
C18:3 ω-3	0.68 ± 0.24b	0.20 ± 0.04a		1.23 ± 0.28c		1.72 ± 0.13d
C21:0		0.13 ± 0.02a			0.39 ± 0.06b	
C20:2ω-6	0.22 ± 0.20a	0.21 ± 0.04a		0.27 ± 0.09a		
C20:3ω-6	0.85 ± 0.49b	0.15 ± 0.02a	1.56 ± 0.26c	0.53 ± 0.25ab	2.01 ± 0.18c	4.57 ± 0.22d
C20:4ω-6	1.43 ± 0.71a					
C20:5 ω-3	0.32 ± 0.27a	0.24 ± 0.04a	0.74 ± 0.08ab	0.93 ± 0.32b	1.39 ± 0.11c	2.35 ± 0.23d
C22:0	0.16 ± 0.08a	2.18 ± 0.50b		1.83 ± 0.21b		
C22:1 ω-9	0.15 ± 0.04a					
C23:0		2.58 ± 0.47c	1.16 ± 0.16a	1.37 ± 0.17a	1.74 ± 0.12ab	2.24 ± 0.24bc
C22:2 ω-6	0.42 ± 0.32a	0.27 ± 0.05a	0.69 ± 0.14a			
C24:0	0.48 ± 0.22a	2.35 ± 0.80c	1.00 ± 0.30ab	0.79 ± 0.20ab	1.18 ± 0.22ab	1.74 ± 0.10bc
C24:1ω-9	0.52 ± 0.20a	0.38 ± 0.07a				
C22:4 ω-6	0.16 ± 0.07a					
C22:5 ω-6	0.22 ± 0.08a	0.29 ± 0.04a			0.68 ± 0.17b	0.53 ± 0.18ab
C22:5 ω-3	0.41 ± 0.20a	0.25 ± 0.08a				
C22:6 ω-3	0.77 ± 0.37a					
SFA	66.79 ± 8.58c	56.99 ± 3.64bc	60.60 ± 4.95bc	50.39 ± 3.45ab	46.98 ± 3.53a	44.62 ± 3.30a
SC-SFA		1.95 ± 0.16a	2.19 ± 0.45a		1.81 ± 0.35a	
MC-SFA	2.26 ± 1.17a	8.26 ± 0.25b	4.76 ± 0.06a	2.21 ± 0.62a	3.64 ± 0.19a	
LC-SFA	64.53 ± 8.65b	46.78 ± 2.58a	53.34 ± 2.10a	48.16 ± 3.57a	41.53 ± 3.05a	44.62 ± 3.30a
MUFA	15.61 ± 3.51a	33.96 ± 2.14 cd	26.33 ± 1.96b	29.59 ± 2.34bc	38.90 ± 2.72d	32.95 ± 1.44 cd
PUFA	17.58 ± 5.53bcd	9.04 ± 0.50a	13.07 ± 0.99ab	20.02 ± 1.11 cd	14.12 ± 1.19abc	22.42 ± 1.14d
PUFA ω-3	2.25 ± 1.38b	0.49 ± 0.04a	2.10 ± 0.33b	2.16 ± 0.15b	1.39 ± 0.11ab	4.07 ± 0.36c
LC-PUFA ω-3	1.41 ± 0.85a	0.49 ± 0.04a	0.74 ± 0.08a	0.93 ± 0.32a	1.39 ± 0.11a	2.35 ± 0.23b
PUFA ω-6	15.36 ± 4.69bc	8.18 ± 0.42a	10.97 ± 0.66ab	17.86 ± 1.06c	12.53 ± 0.84ab	18.35 ± 1.78c
LC-PUFA ω-6	3.15 ± 1.13b	0.92 ± 0.15a	2.25 ± 0.40b	0.80 ± 0.34a	2.69 ± 0.01b	5.10 ± 0.34c
ω-6/ω-3	8.18 ± 4.14a	16.82 ± 2.29b	5.27 ± 0.52a	8.28 ± 0.56a	9.01 ± 0.66a	4.52 ± 0.20a

[a]Means ± SD with the same letter are not significantly different at the 0.05 probability level. [b]SFA, saturated fatty acids; SC-SFA, short-chain SFA; MC-SFA, medium-chain SFA; LC-SFA, long-chain SFA; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFA, long-chain PUFA. [c]Data were cited and reorganized from the results reported by Zou et al.[25]

fats were significantly lower ($P < 0.001$) than that in HMF, whereas when the relative proportions were compared, the SM in donkey milk fat was similar ($P > 0.05$) to that of HMF. The contents of PE in cow, buffalo, sheep, donkey, and camel milk fats were significantly higher ($P < 0.001$) than that in HMF, whereas the contents of PS as well as its relative proportions were significantly lower ($P < 0.001$) than those of HMF. No significant difference ($P > 0.05$) was observed with regard to the contents and relative proportions of PC between cow, buffalo, sheep, donkey, and camel milk fats and HMF. As for PI, cow milk fat had a significantly higher ($P < 0.001$) content than HMF, whereas other mammalian milk fats had significantly lower ($P < 0.001$) contents. When the relative proportions of PI were compared, camel milk fat had a similar ($P > 0.05$) relative proportion to HMF, whereas cow milk fat had significantly higher ($P < 0.001$) and buffalo, sheep, and donkey milk fats had significantly lower ($P < 0.001$) relative proportions.

The fatty acid compositions of phospholipids in human, cow, buffalo, donkey, sheep, and camel milk fats are presented in Table 5. The contents of SFA in phospholipids in donkey, sheep, and camel milk fats were significantly lower ($P < 0.001$) than that in HMF, and no significant difference ($P > 0.05$) was observed in cow and buffalo milk fats. However, in terms of

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LC-SFA, all of the mammalian milk fats had significantly lower ($P < 0.001$) contents than HMF. As for some major LC-SFA species such as C16:0 and C18:0 in phospholipids, some differences between cow, buffalo, donkey, sheep, and camel milk fats and HMF were also observed. The contents of C16:0 in phospholipids of cow, donkey, sheep, and camel milk fats were significantly lower ($P < 0.001$) than that in HMF, and buffalo milk fat had a significantly lower ($P < 0.001$) content of C18:0 in phospholipids than HMF, whereas no significant difference was observed in these of other milk fats. All phospholipids in human, cow, buffalo, donkey, sheep, and camel milk fats had some SFA with carbon number >18 such as eicosanoic acid (C20:0), tricosanoic acid (C23:0), and lignoceric acid (C24:0), which were probably mainly derived from SM.[38] The contents of MUFA in phospholipids of cow, buffalo, donkey, sheep, and camel milk fats were significantly higher ($P < 0.001$) than that in HMF, which was due to the significantly higher ($P < 0.001$) contents of C18:1. Apart from cow milk fat, the PUFA contents of phospholipids in other milk fats had no significant difference ($P > 0.05$) compared with HMF, and the results could also be applied to PUFA ω-3 and PUFA ω-6. As for LC-PUFA, the contents of LC-PUFA ω-3 and ω-6 in phospholipids of camel milk fat were significantly higher ($P < 0.001$), and phospholipids of cow, buffalo, donkey, and sheep milk fats had no significant ($P > 0.05$) difference in LC-PUFA ω-3 contents, whereas cow and donkey milk fats had significantly lower ($P < 0.001$) contents of LC-PUFA ω-6 than HMF. Furthermore, docosahexaenoic acid (C22:6 ω-3) and arachidonic acid (C20:4 ω-6) were not found in phospholipids of cow, buffalo, donkey, sheep, and camel milk fats.

Melting and Crystallization Profiles. The melting and crystallization profiles of milk fat were closely related to their chemical composition. The melting curves are important for investigation of physical existing state of milk fat in the human body. Only the fat with the melting point below the physiological temperature (36.6–37.3 °C) can be quickly emulsified and absorbed. The melting and crystallization curves of human (colostrum, transitional, and mature), cow, buffalo, donkey, sheep, and camel milk fats were investigated (Figure 2). The detected final melting temperatures of colostrum, transitional, and mature milk fat were lower than the physiological temperature. With regard to other mammal animal milk fats, the final melting temperatures of cow, donkey, and sheep milk fats were lower than the physiological temperature, whereas buffalo and camel milk fats had final melting temperatures higher than physiological temperature. Generally speaking, the higher the content of SFA, the higher the melting temperature of milk fat was. However, from the view of fatty acid composition of mammalian milk fats, camel milk fat with the lowest content of SFA had a higher final melting temperature than cow, buffalo, donkey, and sheep milk fats, which had higher contents of SFA. The reason was that the fatty acids composed of SFA in camel milk fat were LC-SFA with high melting point such as C16:0 and C18:0, whereas SC- and MC-SFA in other mammalian milk fats accounted for a large proportion, which decreased melting points. As seen in Figure 2A, most of the components in HMF melted in the middle of the melting range (19.7 ± 2.1, 24.6 ± 1.5, and 20.8 ± 1.7 °C for colostrum, transitional, and mature milk fats, respectively), which might indicate that LC-SFA was scattered in human milk TAGs. However, lots of components in camel, cow, and buffalo milk fats were melted at the end of the melting range (40.5 ± 0.9, 34.8 ± 0.7, 37.8 ± 1.1 °C, respectively),

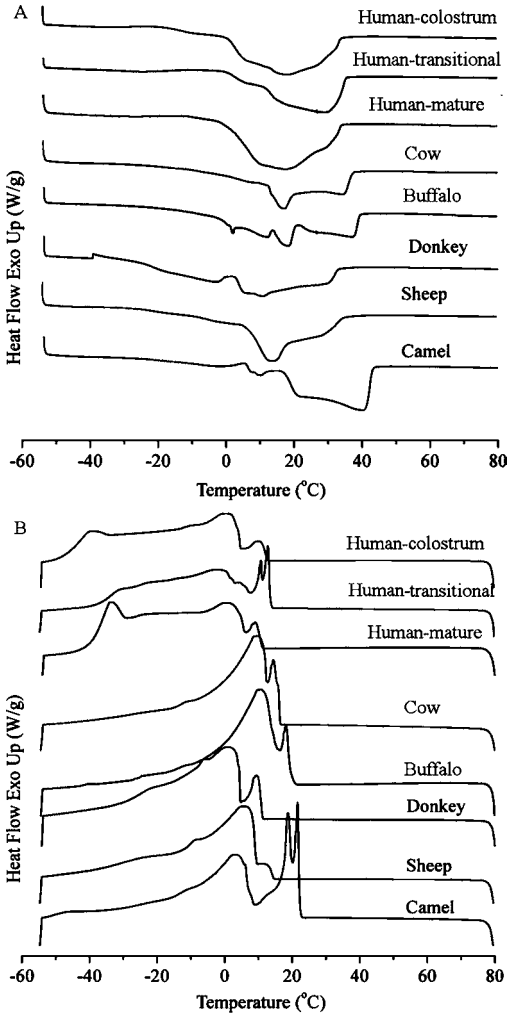

Figure 2. Melting (A) and crystallization (B) profiles of human (colostrum, transitional, and mature), cow, buffalo, donkey, sheep, and camel milk fats.

indicating that these milk fats had some TAGs with high saturation.

Crystallization properties of milk fat, although having little meaning for digestion, are of great importance for application purposes. High starting crystallization temperatures of milk fats corresponded to high final melting temperatures and vice visa. Among these mammalian milk fats, camel milk fat had the highest starting crystallization temperature of 23.1 ± 1.2 °C with a corresponding highest final melting temperature of 42.4 ± 1.5 °C, and donkey milk fat had the lowest starting crystallization temperature of 10.6 ± 0.8 °C with a corresponding lowest final melting temperature of 32.3 ± 1.6 °C.

Similarity Evaluation. The chemical compositions of cow, buffalo, donkey, sheep, and camel milk fats were different from HMF to different degrees based on total and sn-2 fatty acid, TAG, phospholipid, and phospholipid fatty acid compositions, and their difference could be digitized and differentiated by the established model, which considered HMF as the objective; that is, the degree of similarity was 100.[21,22] Therefore, the degrees of similarity of cow, buffalo, donkey, sheep, and camel milk fats to HMF were evaluated and are presented in Table 6. In agreement with the results from the chemical analysis, all of these milk fats had relatively high degrees of similarity in fatty

Table 6. Similarity Evaluation of Cow, Buffalo, Donkey, Sheep, and Camel Milk Fats to Human Milk Fat

similarity	cow	buffalo	sheep	donkey	camel
G_{total}	79.6	74	96.4	84.8	80.2
G_{sn-2}	58.4	59	46.8	44.6	59.6
G_{PUFA}	33.0	32.7	20.2	1.3	15.0
G_{TAG}	36.8	32.1	37.7	51.7	48.3
G_{PL}	71.2	59.1	58.6	68.4	64.1
G_{PL-FA}	61.0	91.5	75.7	61.3	67.8
$G_{PL-PUFA}$	47.9	25.0	34.4	25.4	8.6

acid composition, especially sheep milk fat, whereas the degrees of similarity of other chemical aspects were low, especially sn-2 fatty acid, PUFA, TAG, and PL-PUFA composition. Therefore, none of them meet the requirements of HMFSs. However, considering large amounts of these commercialized mammalian milk fats are good raw materials for infant formula production, it is economical to modify them as HMFSs, and a possible method is physical blending of PUFA and OPO enriched fats and other minor lipids based on the corresponding chemical composition of HMF by precise calculation.[12] As long as the chemical compositions of the blended fats have high degrees of similarity, their melting and crystallization profiles are also similar to those of HMF.

In conclusion, commercial mammalian milk fats including cow, buffalo, donkey, sheep, and camel milk fats were analyzed and compared with HMF on the basis of total and sn-2 fatty acid, TAG, phospholipid, and phospholipid fatty acid compositions and melting and crystallization profiles, which indicated that these milk fats are different from HMF. However, their degrees of similarity could be increased by addition of PUFA and OPO enriched fats and other minor lipids on the basis of the corresponding chemical composition of HMF, thus implying their potential for use as HMFSs.

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Notes

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